THE EFFECT OF LOW STORAGE TEMPERATURE ON REPRODUCTION IN CERTAIN PARASITIC HYMENOPTERA

BY PAUL DE BACH
University of California,
Citrus Experiment Station, Riverside

In population studies involving the rearing of *Musca domestica* L. and its pupal parasites, *Mormoniella vitripennis* (Walker) and *Muscidifurax raptor* Gir., it was found necessary at times to resort to storage by refrigeration in order to synchronize parasite emergence with host production. Storage of adult parasites at low temperatures has, for many years, been practiced by those engaged in biological-control work, both for long-distance shipping of parasites and for proper timing of parasite emergence and liberation. It appears, however, that an adequate general study of the effect of cold storage on insects has never been made, and, as a result, the utility of the method has not been fully realized, especially as regards storage of parasites for any length of time. The fact that results are sometimes adverse has doubtless inhibited investigational work. It is to be hoped that future work will clear up many of the present problems in this important field.

The chief value of cold storage should lie in the *introduction* phase of biological-control work rather than in mass *production*, since the former is concerned with any means of establishing the species, while the latter is more concerned with maximum reproduction after establishment, in order to accomplish complete distribution over the infested area at as early a date as possible. Emphasis should be placed on the fact that in introduction work the percentage mortality or sex-ratio reverses are relatively unimportant so long as sufficient numbers of parasites are sent initially to insure even a few for breeding stock.

Cold Storage of Adult Parasites.—In the present studies, the cold-storage method was found to be quite successful. Parasites were held first as adults, by storage at 4.4° C. Adults of *Mormoniella* and *Muscidifurax*, whose life expectancy in the laboratory is one to two weeks, live as long as five months at this temperature if they are removed at intervals of 3 or 4 days for feeding, although mortality is fairly high after such a long

period. The fecundity of the surviving adults does not appear to be impaired, and the sex ratio of the progeny is favorable. Adults of *Mormoniella vitripennis* and *Muscidifurax raptor* survived five months; adults of *Pachycrepoideus dubius* Ash. and *Microbracon* sp., four months.

Results of tests with *Mormoniella* adults serve to confirm the observations noted above. Four hundred and thirty-three *Mormoniella* reared at 27.5° C. were refrigerated at 4.4° C. from June 6, 1939, to July 13, 1939, or for a total of 37 days. At intervals during this time they were removed from refrigeration and fed honey. The initial age of the paarsites was from 3 to 5 days after emergence. After 37 days of refrigeration, 105 adults remained alive. (Mortality could undoubtedly be decreased by more uniform feeding periods than were used.) These 105 adults were removed from refrigeration and put with housefly puparia in which they were observed to oviposit. From these puparia 289 adults emerged, two-thirds of which were females and one-third males.[1] These data indicate that neither spermatoza nor ova are materially affected by refrigeration of the adult.

The principle of cold storage of insect parasites is, of course, to lower the rate of metabolism so that longevity is increased. This phenomenon is well known, but the exact effects are not well understood. According to Wigglesworth (1939, p. 344),

"In insects exposed to low temperatures, the R. Q. may fall to a very low figure . . . The low temperature seems to cause some disturbance in metabolism, the nature of which is not known. The values at the low temperature are too low to be explained by an exclusive oxidation of fat, and too prolonged to be explained by the increased solubility of gases in the tissue fluids."

Even though the process of cold storage is logically sound, certain complicating factors enter, so that a greater or less amount of mortality occurs, as previously pointed out. Wigglesworth (1939, p. 364) says:

"[Many insects] accustomed to warm surroundings . . . soon die even at temperatures well above freezing-point. The cause of death is not understood. It is often attributed to the accumulation of toxic products which at normal temperatures would be eliminated . . . This type of effect by cold is sometimes termed the 'quantity factor' because it must act for some time before it causes death."

[1] *Mormoniella* exhibits arrhenotokous parthenogenesis.

The mortality obtained in the present study was undoubtedly due to several factors which would be very difficult to analyze separately. Freezing is, of course, a direct cause of mortality but is not to be considered here, since freezing temperatures were not used in these experiments. It should be pointed out, however, that some insects can actually withstand freezing without adverse effects. One possible cause of mortality—one readily thought of—is starvation. Even though parasites are held at a low temperature, a certain amount of metabolism occurs; and, unless the energy lost is regained by periodical feedings and subsequent assimilation of food, death by starvation may result. This is supported by the repeated observation that periodical removal from storage to permit feeding on honey greatly reduced mortality.

A further cause of mortality, sometimes not readily associated with cold storage, may be dessication. Because of the continued removal of moisture in the form of ice, the air in an electric refrigerator of the coil type, such as that used in the present experiments, is very dry. Various authors have shown that low humidity may be a cause of death by means of desiccation. At low temperatures, loss of moisture by the insect would occur very slowly, but this is a factor to be considered, unless parasites are stored in tightly corked vials instead of in those having the usual cotton plug. Van Steenburgh (1934) states that larval parasite mortality during cold storage appears to be due to desiccation of the host egg. And according to Lund (1934, p. 335-36),

" . . . the mortality of the parasites and unparasitized host eggs generally decrease regularly with an increase in humidity at all temperatures—the mortality apparently varying more with humidity than with temperature."

Since the experiments involving cold storage of adult *Mormoniella* and *Muscidifurax* indicated distinct possibilites for this method of shipping refrigerated parasites, it was thought desirable to run tests on storage of their immature stages at low temperatures in order to ascertain whether or not any adverse effects of low storage temperatures on these stages might be evidenced. As a consequence, experiments were designed to test the effect of cold storage on immature stages of the two parasites, *Mormoniella* and *Muscidifurax*.

Cold Storage of Immature Stages.—A common procedure in biological-control work, as is well known, has been that of ship-

ping parasites in the larval or pupal stage in cold storage. Several authors have pointed out recently, however, that low storage temperatures may affect the fecundity of adult parasites thus exposed in their immature stages.

Van Steenburgh's (1934) most satisfactory results with *Trichogramma* pupae in host eggs stored 75 days at 35° to 45° F. showed little mortality but about 50 per cent reduction in fecundity.

Schread and Garman (1934) concluded that

"*Trichogramma* species reared in grain moth eggs are affected by refrigeration in the following ways. (a) At temperatures below 47° F. mortality is gradual and increases with the length of exposure. There is some survival with refrigeration extended to 72 days, but the percentage is so small that it is worthless for production purposes. (b) The sex ratio is upset when temperatures below 47° F. are employed, the change being more evident in the generation following than in the generation emerging from refrigerated eggs. (c) Wing deformity is directly proportional to length of refrigeration and indicates a general weakening of the indivduals."

Under the most adverse conditions tabulated by Schread and Garman (1934), that is, after storage for 39 days at 37° F. and 60 percent relative humidity, the emerging generation exhibited a 1:1 sex ratio, showing that even under such conditions loss of the breeding stock would not result. In an earlier paper, these workers (Schread and Garman 1933) give as the extreme figure a male preponderance of 23:1 in the first generation removed from adults that were subjected to storage at 38° F. and 60 percent relative humidity for 60 days in the pupal stage.

Hanna (1935) found that larvae of the parasite *Euchalcidia caryobori* Hanna were not affected when stored at 16° C. (60° F. +) for as long as 55 days, as shown by normal fecundity and sex ratio of the adults. The preponderance of male progeny from similarly treated pupae, however, indicates an adverse effect in the form of partial or complete sterilization. Male pupae are much more affected than are female. Low storage temperatures cause the retardation of spermatogenesis in the male and possible retardation of the growth of the eggs or ovarian malformation in the female. Flanders (1938) discusses and summarizes the effect of cold storage on the reproduction of parasitic Hymenoptera.

While the aforenamed authors clearly show a reduction in fecundity of adults or a male-predominant sex ratio in certain cases where immature stages of a parasite were subjected to prolonged cold storage, in none of the cases mentioned would this have resulted in loss of the parasite species in introduction, had any quantity of individuals been shipped in storage in both late larval and pupa stages.

The present experiment involved the subjection of full-grown larva of *Mormoniella* to each of the following temperatures: 0-2°, 6°, 10°, 15°, and 23° C. Those held at 23° emerged in 9 days; those held at 15° emerged in 28 days; the remainder did

Table 1.--Progeny of five groups of 50 Mormoniella female parasites which were subjected to storage at different temperatures during the larval stage, then removed and allowed to emerge, mate, and to oviposit, under uniform conditions, among 200 housefly puparia.

Group	Storage		Number of Hosts		Number of Adult Parasites Produced[1]		
	Temperature, in Degrees C.	Period in Days	Stung	Producing Adult Parasites	Male	Female	Total
1	0 - 2	31	120	50	264	238	502
2	6	31	111	47	234	290	524
3	10	31	90	28	117	177	284
4	15	28[2]	21	8	52	41	93
5	23	9[2]	17	5	10	21	31

[1] Mormoniella is a gregarious parasite; as many as 15 may mature in one host.

[2] Adults emerged during storage.

not emerge after being held 31 days, although those at 10° developed into pupae. The larvae held at 0-2° and at 6° showed no outward change. The fact that *Mormoniella* larvae matured and emerged when stored at 23° and 15° and pupated while stored at 10° indicates that the process involved at these three temperatures is nothing more than a slowing down of metabolism, and that development will proceed through to emergence at any temperature above the threshold of development if storage is sufficiently prolonged.

After being held at the different temperatures for the time indicated, the parasite larvae were removed, and the adults were allowed to emerge. Three days later, after feeding and mating had taken place. 50 females of each group were removed and placed with 200 housefly puparia in a quart jar. Oviposition occurred readily, and the parasite progeny matured and emerged in the usual time. These progeny were then counted, and the sex of each was determined. The results of these counts are shown in Table 1.

These data, contrary to what might have been expected from

previous work, indicate that the lower the temperature to which mature larvae of *Mormoniella* are subjected, the greater is the fecundity of the resulting adults. In the present experiments the number of hosts "stung" was considered to be the best measure of fecundity, since an egg was usually deposited when the host was stung. The number of adult parasites that emerged was somewhat variable, partly because of the factor of superparasitization. As a general practice, *Mormoniella* was reared at 27.5° C. in the laboratory. Under such conditions the total progeny approached the numbers obtained at 0-2° and 6° in

Table 2.--Progeny of three groups of 50 Muscidifurax female parasites which were subjected to storage at a temperature of 4.4° C. during immature stages, then removed and allowed to emerge, mate, and to oviposit among 200 housefly puparia.

Group	Stage Tested	Period of Storage at 4.4° C. in Days	Number of Hosts Stung	Number of Adult Parasites Produced		
				Male	Female	Total
1	Larval	28	200	47	70	117
2	Larval	31	200	59	49	108
3	Pupal	25	200	47	40	87
Control[1]			200	30	56	86

[1] Fourteen-day life cycle at 27.5° C.

Table 1. The reason for the low number of progeny obtained from those parasites reared at 15° and 23° is not at all clear, but this was possibly due to their remaining in cold storage for a short time after emergence. These data are of a preliminary nature and must be supplemented in order to determine the exact significance of the effects that have been noted. At least they show plainly that storage at such low temperatures is not harmful so far as reproduction is concerned.

The sex of the progeny of *Mormoniella* adults subjected to different temperatures in their full-grown larval stage, indicates no marked relation between temperature variations and sex ratio, although the data in Table 1 show a slight preponderance of males at 0-2° C. This could be interpreted as indicating that when stored as larvae at a temperature below freezing, males may be affected. The sex ratio for the entire group is 1.13 females to 1.00 male. On the other hand, the sex of adults which have been subjected to low temperatures in the immature stages is about 2.66 females to 1.00 male. Indications are that this may be the effect of differential mortality on the male larvae and pupae.

Experiments similar to those described above were conducted

with *Muscidifurax raptor*, but at only one temperature (4.4° C.). Since pupae had not been used in the previous experiments, they were included here. The results of the tests are given in Table 2. From the data in this table it is evident that even subjection of *Muscidifurax* pupae to prolonged low temperature does not result in the production of sterile adult males (as has been demonstrated for some species), since close to a 1:1 sex ratio was obtained.[2]

There does not appear to be any appreciable difference between experiments and control, either in reproductive capacity or in sex ratio of the progeny, although there are not sufficient data at hand to be evaluated statistically.

Summary and Conclusions.—Mature larvae and pupae of the pteromalid parasites *Mormoniella* and *Muscidifurax* spp. were subjected to storage for periods of 25 to 31 days at temperatures ranging from 0° C. to 23° C.; they were then removed, and the adults were allowed to emerge at a temperature of 27.5° C. Fecundity appeared to be highest in those adults whose immature stages had been subjected to the lowest temperatures. Apparently, immature male parasites were not sterilized at the low temperatures used in these studies, since the succeeding generation exhibited a normal sex ratio. The data obtained show no consistent effect of temperature upon the sex ratio.

Adult *Mormoniella*, *Muscidifurax*, *Pachycrepoideus*, and *Microbracon*, when given periodic feedings, may be held at low temperatures for periods of one to several months without material effect upon their reproductive processes, although mortality increases with length of storage.

In conclusion, it may be stated that, while in certain cases cold storage may, to some degree, affect sex ratios or fecundity, importation of parasites by the cold-storage method, either as adults or as immature stages, may well be feasible and may even be the most satisfactory method of shipment, especially when slow transportation over long distances is necessary. This would at least be the case with the parasites used in the present studies; and, if one may judge by the data of other authors, the method might be applied to many species for introductory purposes. Individual studies, however, will probably be desirable for each species concerned.

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[2] *Muscidifurax* like *Mormoniella*, exhibits arrhenotokous parthenogenesis.

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OBSERVATIONS ON BRACHYSOMIDA CORPULENTA CSY. (Coleoptera; Cerambycidae)[1]

GEO. R. HOPPING
Vernon, British Columbia

Brachysomida corpulenta Csy. was described from a single female from the Levette collection, the type locality given only as California.[2] In the Hopping collection, two females were discovered which agree closely with the original description of *B. corpulenta.* Mr. W. S. Fisher at the U. S. National Museum has kindly compared both specimens with the type in the Casey collection and they agree except in minor detail.

The species is easily recognized by three longitudinal, slightly raised lines on each elytron. These lines are nearly devoid of vestiture and have only a few scattered punctures. The two females before me are dark brown. The type is uniformly brownish-black and the elytra have a slight purplish tinge which seems to be lacking in my specimens. They may not have been fully hardened when they were collected. They were taken by Ralph Hopping at Kaweah, Tulare County, California, over thirty years ago.

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[1] Contribution No. 2247, from the Division of Entomology, Science Service, Department of Agriculture, Ottawa.

[2] Casey, Col. T. L., Memoirs on the Coleoptera 4 :224, 1913.